

**Evaluation of Ozone Injury  
on Vegetation in the  
Moosehorn National Wildlife Refuge  
Maine**

**1999 Observations**

Submitted to

**The U.S. Fish and Wildlife Service  
Air Quality Branch  
Denver, CO**

Donald D. Davis, Ph.D.

March 8, 2000



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## **INTRODUCTION**

### **General**

Moosehorn is one of more than 500 Refuges in the National Wildlife Refuge System (NWRS) administered by the U.S. Fish and Wildlife Service (FWS). The NWRS is a network of lands and waters managed specifically for the protection of wildlife and wildlife habitat and represents the most comprehensive wildlife management program in the world. Units of the system stretch across the United States from northern Alaska to the Florida Keys and include small islands in the Caribbean and South Pacific. The character of the Refuges is as diverse as the nation itself. Moosehorn National Wildlife Refuge (MNWR) was established in 1937 as a refuge and breeding ground for migratory birds and other wildlife. It is the first in a chain of migratory bird refuges that extends from Maine to Florida. The Refuge consists of two units. The Baring Unit covers 16,080 acres and is located off U.S. Route 1 southwest of Calais, Maine. The 6,665-acre Edmunds Unit borders the tidal waters of Cobscook Bay near Dennysville, Maine (Figure 1).

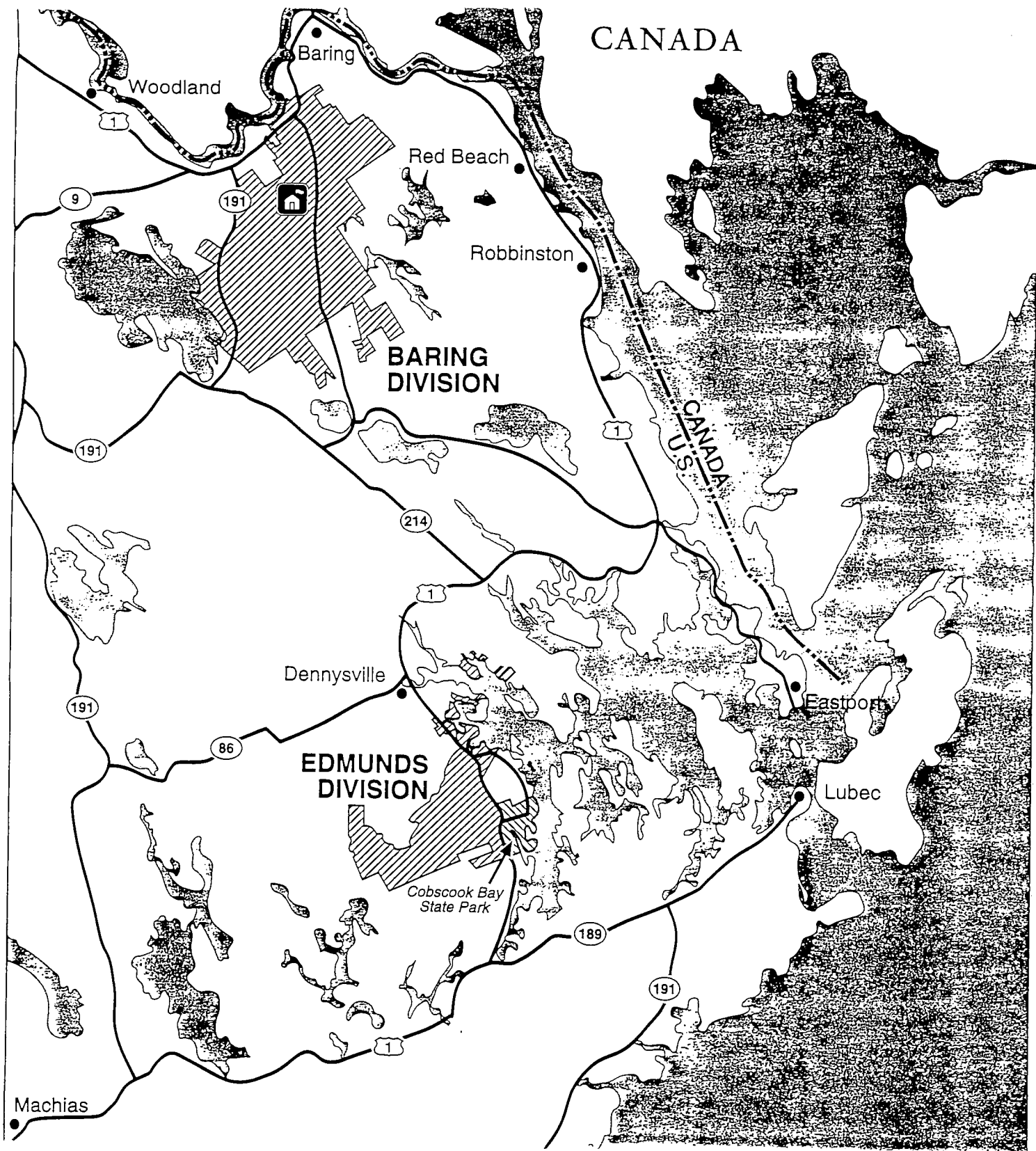
### **Objectives**

- 1). To identify ozone-sensitive plant species in the Moosehorn NWR
- 2). To evaluate the incidence and severity of ozone injury on vegetation in the Moosehorn NWR

### **Justification**

Approximately 4,680 acres of the Baring Unit and 2,780 acres of the Edmunds Unit were set aside by Congress as Wilderness Areas, called the Moosehorn Wilderness. In 1978, Moosehorn Wilderness was designated a Class I air quality area, receiving further protection under the Clean Air Act. Congress gave FWS and the other Federal land managers for Class I areas an "...affirmative responsibility to protect all those air quality related values (including visibility) of such lands..." Air quality related values include vegetation, wildlife, water, soils, visibility, and cultural resources. Despite this special protection, many of the resources in these wilderness areas are being impacted or have the potential to be impacted by air pollutants. Because many air pollutants can be carried long distances in the atmosphere, even rural and remote areas are affected by air pollution, including many wilderness areas. To better understand how air pollution affects resources at the Moosehorn NWR, surveys were conducted in 1998 and 1999 to evaluate ozone injury to vegetation within the refuge.

Figure 1. General map of the Moosehorn National Wildlife Refuge showing location of Baring Unit and Edmunds Unit. The following two figures show location of survey sites in each Unit.



# BARING DIVISION

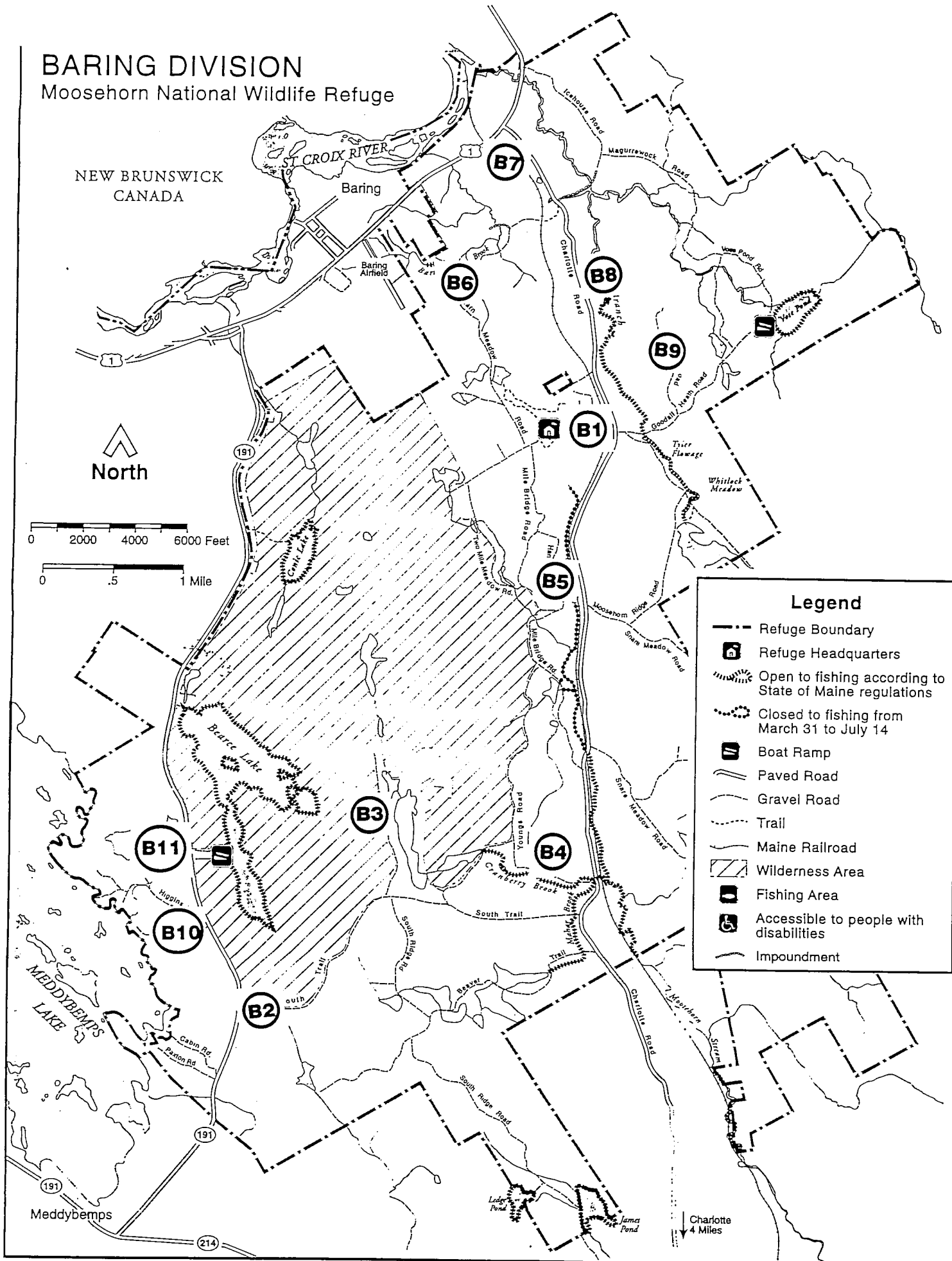
## Moosehorn National Wildlife Refuge

NEW BRUNSWICK  
CANADA



0 2000 4000 6000 Feet

0 .5 1 Mile



### Legend

- Refuge Boundary
- Refuge Headquarters
- Open to fishing according to State of Maine regulations
- Closed to fishing from March 31 to July 14
- Boat Ramp
- Paved Road
- Gravel Road
- Trail
- Maine Railroad
- Wilderness Area
- Fishing Area
- Accessible to people with disabilities
- Impoundment

Charlotte  
4 Miles

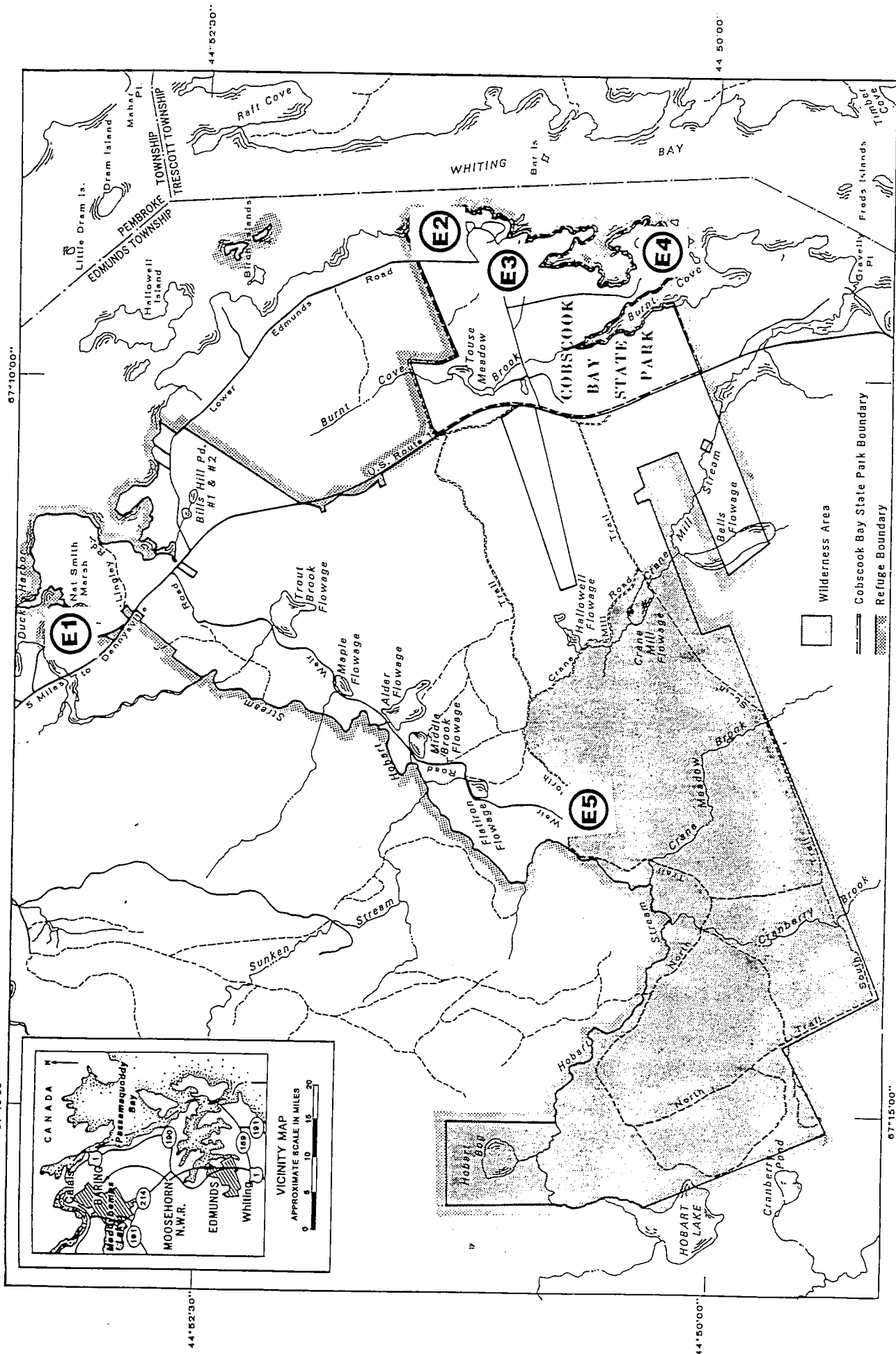
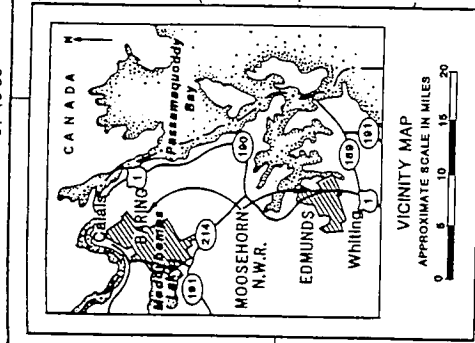


# MOOSEHORN NATIONAL WILDLIFE REFUGE WASHINGTON COUNTY, MAINE

EDMUNDS UNIT  
UNITED STATES  
FISH AND WILDLIFE SERVICE

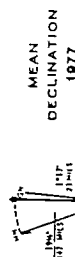
UNITED STATES  
DEPARTMENT OF THE INTERIOR

67°15'00"



67°15'00"

COMPILED IN THE DIVISION OF REALTY  
FROM SURVEYS BY U.S.G.S. and U.S.F.A.W.S.



### **Diagnosis of Air Pollution Injury on Plants**

Although many gaseous air pollutants are emitted into the atmosphere, only certain ones are phytotoxic and capable of injuring plants and inducing symptoms readily apparent during field surveys. The most important of these gaseous, phytotoxic air pollutants are ozone, sulfur dioxide, and fluorides. These pollutants are taken into the plant leaf, along with the normal constituents of the air, through the stomata. Once inside the leaf, the pollutant or its breakdown products react with cellular components, mainly cellular membranes, causing injury or death of tissues.

The resulting macroscopic symptoms, which are visible on the leaf surface, are classified as chronic or acute depending upon the severity of injury. Chronic symptoms imply tissue injury, whereas acute injury signifies tissue death. Chronic symptoms on foliage usually result from exposure of a plant to low levels of pollution for a long time, or occur when a plant is somewhat resistant to a pollutant. Visible ozone injury is usually considered to be chronic injury. Acute injury is observed following a short-term, high concentration of pollution, or occurs when a plant is in a very sensitive condition. Sulfur dioxide injury as observed in the field is often acute. Fluoride injury may be either.

Macroscopic leaf injury caused by air pollutants often represents an intermediate step between initial physiological events and decreases in productivity. Decreases in productivity (Pye 1988) may result in ecological changes, such as reduced diversity (Rosenberg et al. 1979). Visible leaf symptoms induced by phytotoxic pollutants serve as important diagnostic tools that allow observers to identify specific air pollutants as causal agents of vegetation damage (Davis 1984; Skelly et al. 1987). This knowledge can be used in the air pollution emissions permitting process for siting new industries (i.e. Prevention of Significant Deterioration Program), assessment of the secondary air quality standards, assessing the presence of air pollution injury in Class I areas, and in litigation involving air pollution injury.

Although ozone was the air pollutant of concern in this survey, it should be recognized that phytotoxic levels of sulfur dioxide and fluorides can occur near industrial sources, and thus are briefly discussed herein. Likewise, heavy metals (which are not discussed in this report) may be found in excessive levels in vegetation growing in areas downwind from industrial or urban sources (Davis et al. 1984). Toxic heavy metals such as mercury may be of importance in areas being managed for wildlife such as a NWR. However, the presence of excessive heavy metals is

determined with laboratory analysis of foliage, not with surveys dealing with macroscopic foliar injury. Also, environmental biohazards such as dioxins and furans may be found downwind of polluted areas. Although such compounds are of more interest in mammalian and avian toxicity as compared to phytotoxicity, vegetation may absorb or adsorb such contaminants and become part of the contaminated food chain. Laboratory analysis of vegetation for dioxins or furans may serve as part of a biomonitoring effort to determine the levels of these biohazards in a given area.

### Ozone

Ozone is probably the most important and widespread phytotoxic air pollutant in the United States, and is the air pollutant most likely to have an easily recognizable impact on vegetation within a NWR. Background levels of ozone exist naturally in the lower atmosphere, possibly originating from vertical downdrafts of ozone from the stratosphere or from lightning, but more likely from chemical reactions of naturally occurring compounds. However, the major sources of precursors leading to phytotoxic levels of ozone occur within urban areas. In those areas, hydrocarbons and oxides of nitrogen are emitted into the atmosphere from various sources, the most important being automobile exhaust. These compounds undergo photochemical reactions in the presence of sunlight forming photochemical smog, of which ozone is a major component. Smog, ozone, or its precursors may travel downwind for hundreds of miles during long-range transport, depending upon wind direction and movement of weather fronts. Thus, the ozone (or its precursors) impinging on refuges may originate in urban areas many miles from the refuge. Concentrations of ozone are often greater in rural areas downwind from urban areas, as compared to within the urban area itself, due to the presence of reactive pollutants in the urban air which scavenge the ozone.

There are certain bioindicator plants that are very sensitive to ozone (Anderson et al. 1989, Davis and Coppelino 1976, Davis and Skelly 1992, Davis et al. 1981, Davis and Wilhour 1976, and Jensen and Dochinger 1989). The principal investigator in this survey routinely uses the following broad-leaved bioindicator species for evaluating ozone injury: black cherry (Prunus serotina), common elder (Sambucus canadensis), common milkweed (Asclepias syriaca), grape (Vitis spp), white ash (Fraxinus americana), and yellow-poplar (Liriodendron tulipifera). The investigator also uses, but less commonly, Virginia creeper (Parthenocissus quinquefolia) and Viburnum spp. Many of these ozone-sensitive species occur in our refuges in eastern United States.

On broadleaved bioindicators, ozone-induced symptoms usually appear as small, 1 to 2 mm diameter "stipples" of pigmented, black or reddish-purple tissue on the adaxial surface of mature leaves (see Skelly et al. 1987). The pigmented tissue is usually restricted by the veinlets. Immature leaves seldom exhibit symptoms, whereas premature defoliation of mature leaves may occur on sensitive species. To the casual observer, these symptoms are similar to those induced by other stresses (e.g., nutrient deficiency, fall coloration, insects, and certain diseases). However, the pigmented, adaxial stipple on plants of known sensitivity (i.e., black cherry or grape) is a reliable diagnostic symptom of ozone injury.

On eastern conifers, the most reliable symptom (current-year needles only) induced by ozone is a chlorotic mottle, which consists of small patches of chlorotic tissue interspersed within the green, healthy needle tissue. The mottle usually has a "soft edge" (as opposed to a sharply defined edge) to the individual mottled areas. An extremely sensitive plant may exhibit needle tip browning. However, this symptom is common to many stresses and not a reliable diagnostic symptom. Conifer needles older than current-year needles are not useful as monitors, since over-wintering may produce symptoms similar to that caused by ozone. Ozone injury to monocots, such as grasses (i.e., Spartina sp.), is very difficult to diagnose in the field, as there are many causal agents that can result in tipburn and chlorotic mottle on grasses. August and early September are the best times to survey for ozone-induced injury in the Northeast (Davis and Skelly 1992).

### Description of Refuge

The Moosehorn Refuge is a highly glaciated expanse of rolling hills, large ledge outcrops, streams, lakes, bogs, and marshes. The Edmunds Unit has several miles of rocky shoreline where 24-foot tidal fluctuations are a daily occurrence. Approximately 2,780 acres of the Edmunds Unit and 4,680 acres of the Baring Unit were set aside as Wilderness Areas by Congress. As part of the National Wilderness Preservation System these areas are granted special protection that will insure the preservation of their wilderness characteristics.

### Vegetation

The area is rich with history from the logging boom days. In the 1800's horses hauled millions of cords of wood to the shores of the St. Croix River where spring floods carried the logs to Calais mills. Logs were shipped from Calais to world markets by schooner and steamship. However, in the early 1900's, the forest industry began to mechanize and the world market for timber declined. The numerous farms that once were necessary to feed man and beast were abandoned and the forest gradually reclaimed the farmland. A diverse forest of aspen, maple, birch, spruce, and fir currently dominates the landscape and scattered stands of majestic white pine are common.

The refuge is located in terrain that consists of rolling hills with large rock outcrops and scattered boulders. The dominant vegetation in the vicinity of both Units is uneven-aged, second-growth northern conifer-hardwood forest, with some areas in pure spruce-fir. Much of the area was logged and cleared in the 1800s and early 1900s, and several fires have burned over large portions of the area, the last in 1933. Numerous stream valleys, beaver flowages, ericaceous bogs, marshes, and forest/shrub-dominated wetlands occur throughout the area. The deciduous component of the forest includes mixed stands of quaking and bigtooth aspen (Populus tremuloides, P. grandidentata), paper and gray birch (Betula papyrifera, B. populifolia), red maple (Acer rubrum), American beech (Fagus grandifolia), and black cherry (Prunus serotina).

Common understory species include winterberry (Gaultheria procumbens), bracken fern (Pteridium aquilinum), sedges (Carex spp.), and bunchberry (Cornus canadensis). Mixed hardwood-conifer stands occur in many areas, with the generally more shade-tolerant conifers gradually replacing the earlier successional hardwoods. The coniferous component is dominated

by mixed and pure stands of spruce (Picea spp.) and balsam fir (Abies balsamea). Scattered old-growth white pine (Pinus strobus) are an indication of the original climax forest that was present before the lumbering and fires of the last century. Pure stands of alder (Alnus rugosa) are abundant in reverting farmland and wet areas along the margins of streams and beaver flowages. Several blueberry (Vaccinium spp.) fields, meadows, and pastures are maintained as permanent forest openings. In 1976, a long-term management plan was implemented on the refuge to increase the diversity of forest habitat by altering age and species composition, utilizing specific timing of cutting.

Wetlands present in the area include beaver ponds and meadows, marsh, shrub, and forested wetlands of various types, and open-water in the form of streams, ponds and lakes. Beaver meadows in the area are dominated by blue-joint grass (Calamagrostis canadensis) and sedges, with wetter sections and pond fringes supporting marsh plants such as rushes (Juncus spp.), cattail (Typha latifolia), bulrushes (Scirpus spp.), and other non-persistent emergents and aquatic species. Alder and willow (Salix spp.) are common wetland shrubs, and leatherleaf (Chamaedaphne calyculata), sweet gale (Myrica gale), and sphagnum moss (Sphagnum spp.) are dominant bog species. Forested wetlands are dominated by stunted spruce, some white cedar (Thuja occidentalis), red maple, sphagnum, cinnamon fern (Osmunda cinnamomea), and some larch (Larix laricina).

The majority of the Cobscook Bay area is in second growth spruce-fir-pine forest, mixed with some maple, birch, and aspen. There is also some open land, and previously-open land in regrowth stages. The waters adjoining the bay are tidal with fluctuations of up to 24 feet, creating extensive areas of intertidal mudflats in several coves. The tidal range and southern exposures create important ice-free and protected wintering habitat conditions for waterfowl and bald eagles. Many large, old-growth pines are present on uplands adjacent to the shoreline, providing nesting and roosting trees for eagles and other raptors.

A listing of Moosehorn NWR vegetation, as supplied by refuge personnel, is given in the Appendix.

## Wildlife

Moosehorn Refuge is unique among the country's National Wildlife Refuges. Here the American woodcock is intensely studied and managed. This reclusive bird dwells in the alder cover by day and refuge clearings at night. Unfortunately, the Eastern Flyway woodcock population has declined steadily over the past two decades. Research and management programs at Moosehorn have provided valuable information that is being used to stem this decline.

The endangered bald eagle frequents both units of the refuge. In recent years as many as three pairs of eagles have nested at Moosehorn. Eagles are frequently sighted in the area around the Magurrewock Marshes near Route 1 on the Baring Unit and around the tidal waters of Dennys Bay on the Edmunds Unit.

The woodlands of Moosehorn also abounds with many other species. Black bears are abundant and can often be seen along refuge roads in the spring, in the blueberry fields in August, and foraging for apples in the fall. White-tailed deer and an occasional moose feed in the many clearings scattered throughout the refuge. In mid-May a flush of migrating warbles fills the woodlands with song.

The refuge also serves as an important breeding area and migration stop for a variety of waterfowl and other waterbirds. Black ducks, wood ducks, ring-necked ducks, Canada geese, and loons can be seen on the over 50 lakes, marshes, and flowages scattered throughout the refuge. In mid-May the Magurrewock Marsh, which borders U. S. Route 1 on the Baring Unit, abounds with goose and duck broods. Bald eagle sightings also are a common occurrence. Ospreys nest in several of the refuge marshes and the ardent observer can often find river otters frolicking among the cattails. Moosehorn plays an important role in protecting the fragile and diminishing wetland resources of the Atlantic Flyway.

**Management**

Woodcock, ruffed grouse, moose, deer, and a variety of songbirds will only thrive in a young forest. In the past, wildfires periodically rejuvenated the forest. However, wildfire is a rare event today. Forest management programs on the refuge serve to take the place of fire. Small clearcuts scattered throughout the forest provide openings and young brushy growth that serve as food and cover for many wildlife species. This management has resulted in dramatic increases in many species including woodcock, grouse, bear, and moose. Timber harvesting also provides local employment and a percentage of receipts from sales is returned to local communities.

Wetlands management on the refuge has greatly increased waterfowl numbers. Water control structures on the refuge's marshes and ponds allow managers to maintain stable water levels during the breeding season. Water level control also improves the growth of plants that provide food and cover and allows the marshes to be emptied periodically for rejuvenation. The creation of channels, potholes, and islands, as well as shoreline improvement, has also increased waterfowl production and encouraged nesting.



## METHODS

### General Survey Areas

It had been predetermined that survey sites had to occur in open-areas (such as those occurring along roads or trails, or in fields) where ozone-sensitive plant species were found in sunlight and exposed to unrestricted air movement (Anderson et al. 1989; USDA Forest Service, 1990). Immediately prior to the August 1998 survey, the investigator met with refuge personnel. During this time, maps were viewed and discussed which greatly aided and influenced the preliminary selection of survey areas. Based on these initial discussions, tentative survey areas were selected throughout the refuge during 1998. Each area was visited, and its suitability determined. These general areas, with modification, were used in 1999.

### Preliminary Selection of Bioindicator Species

An extensive list of refuge flora was furnished to the investigator by the U.S. Fish and Wildlife Service (Appendix). Prior to the 1998 survey, an initial selection of potential bioindicators that might exhibit ozone injury in the survey area had been selected from this list, as well as by talking to refuge personnel. Plant species or genera on the list that were tentatively selected as bioindicators included: ash (Fraxinus sp.), aster (Aster sp.), black cherry (Prunus serotina), blackberry (Rubus sp.), choke cherry (Prunus virginiana), common milkweed (Asclepias syriaca), elderberry (Sambucus canadensis), mountain ash (Sorbus americana), pin cherry (Prunus pensylvanica), poison-ivy (Rhus radicans = Toxicodendron radicans), serviceberry (Amelanchier laevis), sumac (Rhus sp.), trembling aspen (Populus tremuloides) and viburnum (Viburnum sp.).

Of course, many of the species listed grow in scattered localities through the NWR, and may not be present at designated survey areas; they may only be found with the help of local botanists. Also, it should be pointed out that most plant species growing in the more wet areas of the refuge have not been studied with regard to ozone-induced macroscopic symptoms. That is, the ozone-sensitivity of wetland species, as determined by controlled exposures of ozone, is generally unknown.

### Air Quality

Ozone monitoring data are useful to complement the visual injury surveys. In general, more ozone-induced stipple is likely to occur in years with greater ozone concentrations. However, more consistent and long-term monitoring datasets are needed to further understand the relationship between foliar symptoms, ambient ozone, and environmental conditions (e.g. droughts) in our parks and refuges.

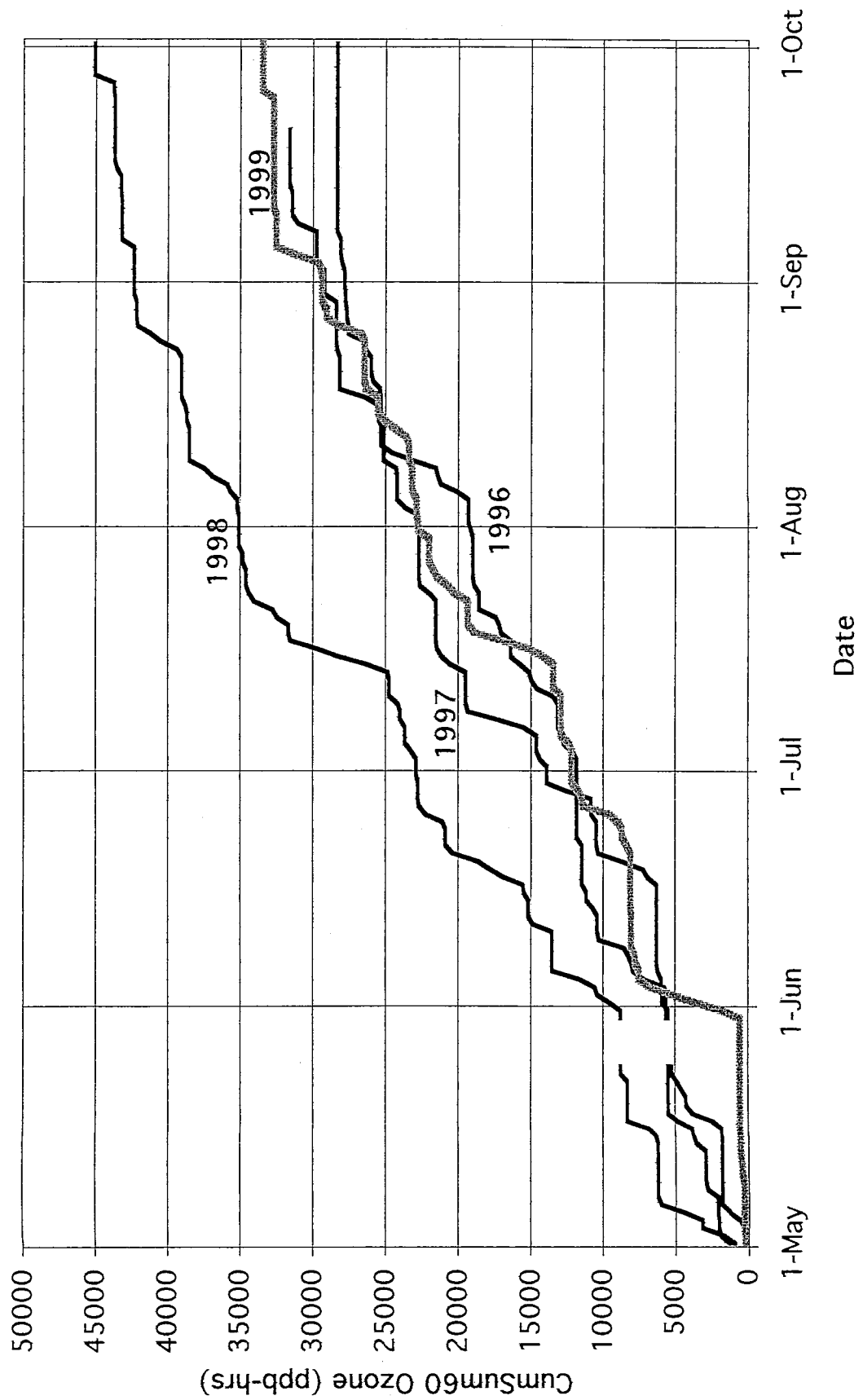
An ozone monitoring site was operating within the Moosehorn NWR (EPA AIRS site #23-029-0019) from 1989 until it closed down in October 1995. The nearest ozone monitor with the most complete datasets is in Acadia National Park at Cadillac Mountain (EPA AIRS site #23-009-0102), located approximately 70 miles southwest of the Moosehorn NWR. The investigator has utilized the EPA ozone database from Cadillac Mountain since 1998.

Ambient ozone levels are expressed as "cumSUM60", the cumulative sum of all hourly ozone concentrations equaling or exceeding 60 ppb. In other studies, we have found that this ozone statistic correlates fairly well with plant damage. During the most recent 4 years (1996-1999) of monitoring at Cadillac Mountain, ozone levels were greatest in 1998 and less in 1996, 1997, and 1999 (Figure 2). Ozone levels in 1996, 1997, and 1999 were similar and considered to be at barely phytotoxic levels (approximately 20,000 ppb-hrs) by August 1. By September 1, the levels (approximately 28,000 ppb-hrs) were definitely phytotoxic. During the last 3 years, the maximum cumSUM60 values peaked at approximately 45,000 ppb-hrs, which occurred in 1998. This is certainly a phytotoxic level of ozone. For comparison to a refuge with extreme ozone concentrations, the ozone levels at the Edwin B. Forsythe NWR near Brigantine, New Jersey, reached about 80,000 ppb-hrs in 1991 (a very high ozone year), and are routinely greater than 40,000 ppb-hrs by the summer's end. During 1999, the Mingo NWR In Missouri) ozone levels likewise reached 80,000 ppb-hrs by Fall.

Assuming that the ozone levels monitored at Cadillac Mountain (70 miles away) are similar to those occurring at the Moosehorn NWR, ozone injury is likely to occur on sensitive species of vegetation within the refuge. However, to my knowledge, there have been no recorded surveys prior to 1998 to document whether or not ozone injury has occurred on refuge vegetation.

Figure 2. Cumulative sum of all hourly ozone concentrations equaling or exceeding 60 ppb (cumSUM60) monitored at Acadia National Park, Cadillac Mountain, Maine (EPA AIRS Site # 23-009-0102) during 1996-1999; units are ppb-hrs. This monitoring site is located approximately 70 miles southwest of the Moosehorn refuge.

# Ozone Levels at Acadia, ME



### Surveys Dates and Areas

The Moosehorn National Wildlife Refuge was surveyed on July 22-25 in 1999. During 1998, it had been surveyed twice, on July 29-August 2 and August 25-28. The earlier date was chosen in 1999 because of the widespread drought that occurred in the East during late summer. The investigator felt that a survey conducted later in the summer of 1999 would be greatly confounded by a severe drought. (Based on these experiences, the best time to survey this refuge in the future is probably late-August).

As described earlier, the tentative location of survey areas was based on discussions with refuge personnel and examination of maps at the refuge headquarters prior to the first 1998 survey. These areas were then visited on-site during the 1998 and 1999 surveys, and modifications made. Approximately 16 sites were deemed suitable for ozone injury surveys, based on openness, accessibility, and presence of bioindicators (Figure 1). All 16 sites were visited in 1999, but data was not necessarily taken at all sites. In addition to these 16 specific areas, vegetation was observed as the investigator traveled from site to site during both 1998 and 1999.

### Severity Rating

Each broadleaved plant evaluated for ambient ozone injury had to have foliage within reach; that is, trees were not climbed nor were pole-pruners used. The ForestHealth Expert System had been used to train the investigator in estimating the amount of stipple on a leaf. For broadleaved tree species, the percentage of ozone injury was estimated on the oldest leaf on each of four branches, and the average value recorded. Then, the next oldest leaf was evaluated, and so on, until the five oldest leaves had been rated. For each herbaceous plant, each of the five (if present) oldest (basal) leaves of the plant was examined and the average percent stipple recorded. Each of the oldest five leaves on the current woody growth (canes) of vines was rated and the average percent stipple recorded. On all species, only adaxial leaf surfaces were evaluated. Symptom severity on the adaxial surface of each leaf evaluated was estimated by assigning severity classes, based on the percentage of surface injured, of 0, 5, 10, 20, 40, 60, 80, 90, 95 and 100 %. Photographs (slides) were taken. One slide set will remain with the investigator, and one set will be sent to the FWS Air Quality Branch in Denver.

## RESULTS AND DISCUSSION

### Final Selection of Bioindicator Species

A more complete selection of bioindicator species or genera (plants regarded as being sensitive to ozone) was made on-site during the two visits of 1998. During the first 1998 visit to the refuge, it was immediately noted that spreading dogbane (Apocynum androsaemifolium) was exhibiting adaxial stipple, typical of that caused by ozone, at several sites. Therefore, dogbane was added to the bioindicator list, which then consisted of ash (mainly white ash), black cherry, blackberry, choke cherry, pin cherry, serviceberry, and trembling aspen as bioindicator species. These were among the most common of the ozone-sensitive species in the refuge, and usually occurred in open areas. Not all species/genera listed were present at all sites. During the 1998 and 1999 on-site surveys, this list was amended to also include sand cherry (Prunus pumila), raspberry (Rubus idaeus), and sarsaparilla (Aralia nudicaulis). The latter two species, along with blackberry, were selected as indicators for sulfur dioxide injury. However, since there was no point source of SO<sub>2</sub> readily evident, emphasis was placed on the ozone-sensitive bioindicators.

Most plant species in the wetlands of the Moosehorn refuge have not been carefully studied with regard to ozone-induced macroscopic symptoms. That is, the ozone-sensitivity of wetland plants as determined by controlled exposures of ozone is generally unknown, and it is unwise to attribute ozone as the cause of various foliar discoloration on untested species. However, it was deemed important to evaluate even unknown species, because of their possible importance in the wetlands ecosystem, for the presence or absence of ozone-induced dark adaxial stipple. In this regard, stipple was not observed in 1998-1999 on any known or unknown species of wetland plants in the Moosehorn refuge. Assuming that ozone-sensitive wetland plants exist in the refuge, this lack of symptoms may have been related to the low levels of ozone in 1998, or to the dry conditions that existed in the refuge during 1999. During dry conditions, stomatal closure occurs, limiting ozone uptake and subsequent ozone injury. Of course, since the investigator was concentrating on the well-known, upland indicators, he may have overlooked any stipple on the foliage of plants growing in the wetter areas.

## Foliar Symptoms

### Baring Unit

**Site B1 (Refuge Headquarters).** Ozone-induced injury was observed during the 1999 visit (July 22-25) only at sites B1 and B8 (one plant) within the Baring Unit (Table 1). During the August 1998 survey, ozone injury had been noted at sites B1, B2, and B5 within this unit. At Site B1, vegetation was examined in the large, open fields along the entrance road leading to the refuge headquarters (Figure 1, Location B1). There were several bioindicator "species" present in these large openings at site B1; this is considered to be an excellent survey site.

Ozone injury was light in both incidence and very light (trace) in severity on spreading dogbane plants (in flower), where injury was present on 9 of 113 (8.0%) plants examined (Table 1). As in 1998, the ozone injury on dogbane was restricted to older paired leaves on the primary stems, and did not occur on the pairs of leaves further out the secondary shoots. (Secondary shoots arose from the axil of these pairs of primary stem leaves). The severity of injury on the two symptomatic leaves was judged to be very light, usually involving less than 5% of leaf area, and was less severe than in August 1998.

Near the upper edge of one field, brush and grass had been mowed, and very succulent aspen shoots had sprouted. It is my experience that fast-growing, succulent shoots often show more foliar ozone injury, as compared to the leaves on larger trees or on slower growing seedlings or shoots. Ozone injury was present on 6 of 58 (10.3%) of the aspen in this area; injury was restricted to the fast-growing sprouts, and did not occur on the mature trees or saplings. The severity of the injury on the six plants was judged to be very light (Table 2). Ozone injury on aspen did not occur at this site in 1998.

Table 2. Severity of ozone-induced injury on leaves of symptomatic leaves of aspen at Site B1.

Species	Plant No.	Leaf Number				
		1*	2	3	4	5
<b>Aspen</b>	1	5	10	10	5	5
	2	10	20	20	5	5
	3	10	20	20	5	5
	4	5	10	10	5	0
	5	10	10	5	0	0
	6	10	5	10	0	0

\*Oldest leaf of the 5 leaves evaluated.

\*\*Severity values = 0, 5, 10, 20, 40, 60, 80, 90, 95, and 100% of leaf tissue injured.

Table 1. Summary of observations made during the 1999 survey at the Moosehorn National Wildlife Refuge. Numbers in table refer to number of plants with ozone-induced injury as compared to the total number of plants evaluated for that species, and expressed as percentages. Comparison is made with August 1998 results.

Date/Site	Ash	Aspen	Black Cherry	Black-berry	Choke Cherry	Spread. Dogbane	Pin Cherry	Service-berry	Rasp-berry	Sand Cherry	Min Ash	Sarsap-arilla
(July 29-Aug. 2)												
<b>Baring Unit</b>												
B1		6/58	0/20	0/40	0/200	9/113	0/10	0/8				
B2	2/40											
B3	0/20	0/20										
B4		0/5	0/30	0/30		0/30	0/30					
B5												
B6												
B7												
B8	0/9			0/30	0/20		0/1		0/30			
B9	0/8	0/50	0/9	0/50	0/20				0/40			
B10												
B11												
<b>Edmunds Unit</b>												
E1		0/15		0/40		1/30	0/20	0/35		0/40		
E2			5/26				3/13		0/20			
E2a			0/26				0/13		0/20		0/10	0/10
E3		1/20		0/100	0/20	0/5	1/5	1/6	0/100			
E4		2/198		0/20			0/67					
E5		0/30		0/20			0/37		0/20			
1999 Total	2/77	9/396	5/111	0/330	0/260	10/178	4/196	1/49	0/230	0/40	0/10	0/10
1999 %	2.6%	2.3%	4.5%	0.0%	0.0%	5.6%	2.0%	2.0%	0.0%	0.0%	0.0%	0.0%
Aug 98 Total	1/54	10/126	0/55	0/53	0/30	7/77	28/108					
Aug 98 %	1.8%	7.9%	0.0%	0.0%	0.0%	9.0%	25.9%					



Ozone injury was not observed on black cherry, blackberry, choke cherry, pin cherry, or serviceberry. Although choke cherry was evaluated, the investigator considers this species to be tolerant to ambient levels of ozone and it may not be evaluated in the future. There was a slight reddening, which may or may not have been related to ozone injury, on blackberry. Leafspots were common on dogbane and serviceberry. Choke cherry sprouts had very severe powdery mildew infections. Black cherry had black knot galls on the branches and spindle galls on the leaves. This low level of ozone-induced symptoms, at this and other sites, was attributed to the low levels of ambient ozone (Figure 2) present in this area prior to this 1999 survey, as well as being influenced by the dry weather that preceded the survey.

**Sites B2-B11 (see Table 1).** At site B2 (western end of South Trail) 2 of 40 (5.0%) of the ash seedlings and saplings examined showed classic ozone injury. At site B3 (Cranberry Lake Inlet) none of the 20 ash nor the 20 aspen had ozone injury. Plants at site B4 (Cranberry Brook) likewise showed a lack of ozone injury. At site B5 (Hanson Soil Pit Road), many of the aspen sprouts were severely stressed by a severe fungal leaf and shoot blight, likely caused by the fungus *Venturia*. Plants were not evaluated because of this confounding factor. Few good indicator plants were found at sites B6 (Barn Meadows #2) and B7 (Powerline at the intersection of Route 1 and Charlotte Road); these two sites may be eliminated. Site B8 (West Branch Observation Deck) is also marginal site in terms of usefulness, but is better than B6 and B7; ozone injury was not observed at site B8. Site B9 (Road to old Air Monitoring Station) was expanded in 1999 to include Voss Pond. This is now an excellent site. However, ozone injury was not present, and *Venturia* shoot blight was present in moderate amounts on aspen.

Sites B6, B7, B10 (Higgins Road) and B11 (Bearce Flowage) are considered to be marginal survey sites. Sites B6, B7, and B10 were not used in 1998 because few bioindicators were present; however, all four sites will be maintained and revisited briefly in future years, as they are very open areas. The area around the Bearce Boat Launch will be investigated more thoroughly in the future, as it was noted that ash trees were present in the general area.

### Edmunds Unit

**Site E1.** Vegetation was examined in 1999 in and around the edges of the farm fields near the Nate Smith Marsh. As in 1998, there were several bioindicators present in these large fields. Overall, ozone injury on ozone-sensitive plants was extremely light in both incidence and severity, occurring only on one spreading dogbane plant of 30 (3.3%) plants examined (Table 1). The severity on ozone injury on this one plant was very light, affecting about 5% leaf area on symptomatic leaves. Again, ozone injury on dogbane was restricted to the paired leaves on the stems, and did not occur on the other leaves. Injury was not observed on blackberry, pin cherry, sand cherry, serviceberry, or trembling aspen. Black knot disease was very severe on Prunus at this location.

**Site E2.** Ozone injury was not observed on black cherry or pin cherry plants along the boat launch in Cobscook State Park (Table 1). Raspberry plants had healthy leaves at this site. Numerous indicator plants were noted at the nearby boat launch. This site was designated as SiteE2a. There was no ozone injury on any bioindicators examined (Table 1). Birch trees had severe leaf miner injury. Mountain ash trees had severe leafhopper-type injury, which confounded ozone injury evaluations on this species. Here and elsewhere in the area, there was no SO<sub>2</sub> injury on birch, raspberry, blackberry, nor sarsaparilla.

**Site E3.** Bioindicators were examined in and around the edge of this very large field within the Cobscook State Park, near the edge of Whiting Bay. Ozone injury was noted on 5 of 26 (19.2%) of the black cherry and 3 of 13 (23.1%) of the pin cherry plants examined. Raspberry plants had healthy leaves.

**Site E4.** Vegetation was examined in this very large field near the south end of Cobscook State Park. There was a large clump of pin cherry saplings in the field. These plants were possibly from the same seed source, or sprouts, since they were growing close together. In 1998 19 of 40 (47.5%) pin cherries in this clump examined exhibited ozone injury. However, the clump had been recently cut in 1999, and stumps were just beginning to sprout. A total of 67 new sprouts were examined, but ozone injury was not observed (Table 1). It is likely that the pin cherry leaves were not mature enough in 1999 to exhibit ozone injury, or they were so new that they had not been exposed to sufficient levels of ozone to cause injury. There was, however, ozone injury to 2 of 198 aspen sprouts in the field. Ozone injury was not present on blackberry leaves.

**Site E5.** Thirty-seven pin cherries were examined near the intersection of North Trail and Weir Road. There was no ozone injury on the pin cherries, aspen, or blackberry. Pin cherry had a severe leafspot disease. Raspberry leaves were healthy.

## SUMMARY

The results of this 1999 survey revealed that ozone injury was present on vegetation within the boundaries of the Moosehorn NWR, a portion of which is a Class I air quality area. Prior to this visit, this part of Maine had experienced quite low ozone levels (Figure 2), as measured at the Acadia National Park monitoring site. The 1999 ozone levels were much lower than in 1998, which in retrospect had fairly high ozone levels. The overall incidence and severity of observed ozone injury was, accordingly, extremely light during the 1999 survey. In 1998, ozone-induced foliar symptoms were greater, especially during the second visit, due to the high phytotoxic levels of ozone that occurred in the time period between the two visits of 1998.

In addition to the light ozone-induced symptoms, other common plant disorders observed in the refuge in 1999 included leafspots on serviceberry (severe), cherries, aspen, white birch, and hawthorn. Cankers were present on birch, and black knot galls were common on cherries. Insect disorders included skeletonizers and webworm on alder, and leaf miner on white birch. Incipient drought symptoms were widespread, and included yellowing, wilting, curling and defoliation. Fruits and berries in various plants were small due to the lack of water. It is very likely that this dry weather precluded ozone uptake by plants, and reduced subsequent symptom development.

The Moosehorn NWR should be surveyed in again (third survey) in 2000 to document the presence or absence of ozone-induced injury on vegetation over time. It is recommended that the survey take place in late August. These results should prove useful to the FWS when making air quality management decisions, including those related to the review of Prevention of Significant Deterioration (PSD) permits.

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*Carex trisperma* (threeseeded sedge)  
*Carum carvi* (caraway)  
*Cerastium vulgatum* (*Cerastium fontanum* ssp. *vulgare*)  
*Chamaedaphne calyculata* (leatherleaf)  
*Chelone glabra* (white turtlehead)  
*Chimaphila umbellata* (pipsissewa)  
*Chrysanthemum leucanthemum* (*Leucanthemum vulgare*)  
*Circaea alpina* (small enchanter's nightshade)  
*Cirsium arvense* (Canadian thistle)  
*Cirsium discolor* (field thistle)  
*Cirsium vulgare* (bull thistle)  
*Clematis virginiana* (devil's darning needles)  
*Clintonia borealis* (yellow bluebeadlily)  
*Comptonia peregrina* (sweet fern)  
*Coptis groenlandica* (*Coptis trifolia* ssp. *groenlandica*)  
*Cornus alternifolia* (alternateleaf dogwood)  
*Cornus canadensis* (bunchberry dogwood)  
*Cornus stolonifera* (*Cornus sericea* ssp. *sericea*)  
*Coronilla varia* (purple crownvetch)  
*Corylus cornuta* (beaked hazelnut)  
*Crassula aquatica* (water pygmyweed)  
*Cycloloma atriplicifolium* (winged pigweed)  
*Cypripedium acaule* (pink lady's slipper)  
*Cypripedium calceolus* ()  
*Dactylis glomerata* (orchardgrass)  
*Dalibarda repens* (robin runaway)  
*Danthonia spicata* (poverty danthonia)  
*Dennstaedtia punctilobula* (eastern hayscented fern)  
*Deschampsia flexuosa* (wavy hairgrass)  
*Dianthus armeria* (Deptford pink)  
*Diervilla lonicera* (northern bush honeysuckle)  
*Distichlis spicata* (inland saltgrass)  
*Dryopteris cristata* (crested woodfern)  
*Dryopteris disjuncta* (*Gymnocarpium disjunctum*)  
*Dryopteris marginalis* (marginal woodfern)  
*Dryopteris noveboracensis* (*Thelypteris noveboracensis*)  
*Dryopteris phegopteris* (*Phegopteris connectilis*)  
*Dryopteris spinulosa* (*Dryopteris carthusiana*)  
*Dryopteris thelypteris* (*Thelypteris palustris* var. *pubescens*)  
*Dulichium arundinaceum* (threeway sedge)  
*Epigaea repens* (trailing arbutus)  
*Epilobium angustifolium* (fireweed)  
*Equisetum arvense* (field horsetail)  
*Equisetum fluviatile* (water horsetail)  
*Equisetum hyemale* (scouringrush horsetail)  
*Equisetum sylvaticum* (woodland horsetail)

*Erigeron annuus* (eastern daisy fleabane)  
*Erigeron strigosus* (prairie fleabane)  
*Eriocaulon septangulare* (*Eriocaulon aquaticum*)  
*Eriophorum angustifolium* (tall cottongrass)  
*Eriophorum spissum* (*Eriophorum vaginatum* var. *spissum*)  
*Eupatoriadelphus purpureus* (*Eupatorium purpureum* var. *purpureum*)  
*Eupatorium maculatum* (spotted joe pyeweed)  
*Euthamia graminifolia* (flattop goldentop)  
*Fagus grandifolia* (American beech)  
*Festuca capillata* (*Festuca filiformis*)  
*Festuca elatior* (*Festuca pratensis*)  
*Festuca rubra* (red fescue)  
*Fragaria virginiana* (Virginia strawberry)  
*Fraxinus americana* (white ash)  
*Fraxinus pennsylvanica* (green ash)  
*Galeopsis tetrahit* (brittlestem hempnettle)  
*Galium mollugo* (false baby's breath)  
*Galium palustre* (common marsh bedstraw)  
*Galium triflorum* (fragrant bedstraw)  
*Gaultheria hispidula* (creeping snowberry)  
*Gaultheria procumbens* (eastern teaberry)  
*Gaylussacia baccata* (black huckleberry)  
*Gaylussacia dumosa* (dwarf huckleberry)  
*Geranium bicknellii* (Bicknell's cranesbill)  
*Glyceria canadensis* (rattlesnake manna grass)  
*Glyceria obtusa* (Atlantic manna grass)  
*Hamamelis virginiana* (American witchhazel)  
*Hemerocallis fulva* (orange daylily)  
*Hieracium aurantiacum* (orange hawkweed)  
*Hieracium florentinum* (*Hieracium piloselloides*)  
*Hieracium pilosella* (mouseear hawkweed)  
*Hieracium pratense* (*Hieracium caespitosum*)  
*Hordeum californicum* (California barley)  
*Humulus lupulus* (common hop)  
*Hypericum ellipticum* (pale St. Johnswort)  
*Hypericum perforatum* (common St. Johnswort)  
*Ilex verticillata* (common winterberry)  
*Impatiens capensis* (jewelweed)  
*Iris versicolor* (harlequin blueflag)  
*Juniperus communis* (common juniper)  
*Kalmia angustifolia* (sheep laurel)  
*Kalmia polifolia* (bog laurel)  
*Larix laricina* (tamarack)  
*Ledum groenlandicum* (bog Labrador tea)  
*Lilium canadense* (Canadian lily)  
*Linaria canadensis* (*Nuttallanthus canadensis*)



*Linaria vulgaris* (butter and eggs)  
*Linnaea borealis* (twinflower)  
*Lobelia cardinalis* (cardinalflower)  
*Lobelia dortmanna* (Dortmann's cardinalflower)  
*Lonicera canadensis* (American fly honeysuckle)  
*Ludwigia palustris* (marsh seedbox)  
*Luzula acuminata* (hairy woodrush)  
*Luzula multiflora* (common woodrush)  
*Lychnis alba* (*Silene latifolia* ssp. *alba*)  
*Lycopodium annotinum* (stiff clubmoss)  
*Lycopodium clavatum* (running clubmoss)  
*Lycopodium complanatum* (groundcedar)  
*Lycopodium lucidulum* (*Huperzia lucidula*)  
*Lycopodium obscurum* (rare clubmoss)  
*Lycopodium tristachyum* (deeproot clubmoss)  
*Lycopus americanus* (American waterhorehound)  
*Lycopus uniflorus* (northern bugleweed)  
*Lycopus virginicus* (Virginia waterhorehound)  
*Lysimachia terrestris* (earth loosestrife)  
*Maianthemum canadense* (Canada beadruby)  
*Matricaria matricarioides* (*Matricaria discoidea*)  
*Medeola virginiana* (Indian cucumberroot)  
*Medicago sativa* (alfalfa)  
*Melampyrum lineare* (narrowleaf cowwheat)  
*Melilotus alba* (white sweetclover)  
*Melilotus officinalis* (yellow sweetclover)  
*Mentha arvensis* (*Mentha canadensis*)  
*Mimulus ringens* (ringen monkeyflower)  
*Mitchella repens* (partridgeberry)  
*Moneses uniflora* (single delight)  
*Myrica gale* (sweetgale)  
*Myriophyllum exalbescens* (*Myriophyllum sibiricum*)  
*Nuphar variegata* (*Nuphar lutea* ssp. *variegata*)  
*Nymphaea odorata* (American white waterlily)  
*Nymphoides cordata* (little floatingheart)  
*Odontites serotinus* (*Odontites vernus* ssp. *serotinus*)  
*Oenothera biennis* (common eveningprimrose)  
*Oenothera fruticosa* (narrowleaf eveningprimrose)  
*Onoclea sensibilis* (sensitive fern)  
*Oryzopsis asperifolia* (roughleaf ricegrass)  
*Osmunda cinnamomea* (cinnamon fern)  
*Osmunda claytoniana* (interrupted fern)  
*Osmunda regalis* (royal fern)  
*Oxalis europaea* (*Oxalis stricta*)  
*Oxalis montana* (mountain woodsorrel)  
*Oxalis stricta* (common yellow oxalis)

*Phalaris arundinacea* (reed canarygrass)  
*Phleum pratense* (timothy)  
*Picea abies* (Norway spruce)  
*Picea glauca* (white spruce)  
*Picea mariana* (black spruce)  
*Picea rubens* (red spruce)  
*Pinus resinosa* (red pine)  
*Pinus strobus* (eastern white pine)  
*Plantago major* (common plantain)  
*Pogonia ophioglossoides* (snakemouth orchid)  
*Polygonatum pubescens* (hairy Solomon's seal)  
*Polygonum amphibium* (water knotweed)  
*Polygonum careyi* (Carey's smartweed)  
*Polygonum cilinode* (fringed black bindweed)  
*Polygonum lapathifolium* (curlytop knotweed)  
*Polygonum pensylvanicum* (Pennsylvania smartweed)  
*Polygonum punctatum* (dotted smartweed)  
*Polypodium virginianum* (rock polypody)  
*Polystichum acrostichoides* (Christmas fern)  
*Pontederia cordata* (pickerelweed)  
*Populus balsamifera* (balsam poplar)  
*Populus grandidentata* (bigtooth aspen)  
*Populus tremuloides* (quaking aspen)  
*Potamogeton epihydrus* (ribbonleaf pondweed)  
*Potamogeton natans* (floating pondweed)  
*Potamogeton pectinatus* (sago pondweed)  
*Potamogeton zosteriformis* (flatstem pondweed)  
*Potentilla anserina* (Argentina anserina)  
*Potentilla argentea* (silver cinquefoil)  
*Potentilla norvegica* (Norwegian cinquefoil)  
*Potentilla recta* (sulphur cinquefoil)  
*Potentilla simplex* (common cinquefoil)  
*Prunella vulgaris* (common selfheal)  
*Prunus pensylvanica* (pin cherry)  
*Prunus pumila* var. *besseyi* (western sandcherry)  
*Prunus serotina* (black cherry)  
*Prunus virginiana* (common chokecherry)  
*Pteridium aquilinum* (western brackenfern)  
*Pyrola elliptica* (waxflower shinleaf)  
*Pyrola rotundifolia* (*Pyrola americana*)  
*Pyrus americana* (*Sorbus americana*)  
*Pyrus floribunda* (*Aronia X prunifolia*)  
*Pyrus malus* (*Malus sylvestris*)  
*Pyrus melanocarpa* (*Aronia melanocarpa*)  
*Quercus rubra* (northern red oak)  
*Ranunculus acris* (tall buttercup)

*Rhinanthus crista-galli* (*Rhinanthus minor* ssp. *minor*)  
*Rhododendron canadense* (*rhodora*)  
*Rhus radicans* (*Toxicodendron radicans* ssp. *radicans*)  
*Rhus typhina* (*Rhus hirta*)  
*Ribes glandulosum* (skunk currant)  
*Ribes hirtellum* (hairystem gooseberry)  
*Rubus allegheniensis* (Allegheny blackberry)  
*Rubus hispidus* (bristly dewberry)  
*Rubus idaeus* (American red raspberry)  
*Rubus pubescens* (dwarf red blackberry)  
*Rudbeckia serotina* (*Rudbeckia hirta* var. *pulcherrima*)  
*Rumex acetosella* (common sheep sorrel)  
*Ruppia maritima* (widgeongrass)  
*Sagittaria latifolia* (broadleaf arrowhead)  
*Salicornia europaea* (*Salicornia maritima*)  
*Salix bebbiana* (Bebb willow)  
*Salix gracilis* (*Salix petiolaris*)  
*Sambucus canadensis* (American elder)  
*Sarracenia purpurea* (purple pitcherplant)  
*Scirpus atrovirens* (green bulrush)  
*Scirpus cyperinus* (woolgrass)  
*Scirpus pedicellatus* (stalked bulrush)  
*Scirpus rubrotinctus* (*Scirpus microcarpus*)  
*Scutellaria epilobiifolia* (*Scutellaria galericulata*)  
*Scutellaria lateriflora* (blue skullcap)  
*Sedum purpureum* (*Sedum telephium* ssp. *telephium*)  
*Senecio aureus* (golden ragwort)  
*Senecio vulgaris* (common groundsel)  
*Silene antirrhina* (sleepy silene)  
*Silene cucubalus* (*Silene vulgaris*)  
*Sisyrinchium montanum* (mountain blueeyed grass)  
*Sium suave* (hemlock waterparsnip)  
*Smilacina racemosa* (*Maianthemum racemosum* ssp. *racemosum*)  
*Smilacina trifolia* (*Maianthemum trifolium*)  
*Solanum dulcamara* (climbing nightshade)  
*Solidago graminifolia* (*Euthamia graminifolia* var. *graminifolia*)  
*Spiraea latifolia* (*Spiraea alba* var. *latifolia*)  
*Spiraea tomentosa* (steeplebush)  
*Stellaria graminea* (grasslike starwort)  
*Taraxacum officinale* (common dandelion)  
*Taxilejeunea* (*taxilejeunea*)  
*Thalictrum polygamum* (*Thalictrum pubescens*)  
*Thelypteris thelypteroides* (*Thelypteris noveboracensis*)  
*Thuja occidentalis* (eastern arborvitae)  
*Tragopogon pratensis* (meadow salsify)  
*Trientalis borealis* (American starflower)

*Trifolium agrarium* (*Trifolium aureum*)  
*Trifolium arvense* (rabbitfoot clover)  
*Trifolium hybridum* (alsike clover)  
*Trifolium pratense* (red clover)  
*Trifolium repens* (white clover)  
*Tsuga canadensis* (eastern hemlock)  
*Typha angustifolia* (narrowleaf cattail)  
*Typha latifolia* (broadleaf cattail)  
*Utricularia cornuta* (horned bladderwort)  
*Utricularia purpurea* (eastern purple bladderwort)  
*Utricularia vulgaris* (*Utricularia macrorhiza*)  
*Uvularia sessilifolia* (sessileleaf bellwort)  
*Vaccinium angustifolium* (lowbush blueberry)  
*Vaccinium corymbosum* (highbush blueberry)  
*Vaccinium macrocarpon* (cranberry)  
*Vaccinium myrtilloides* (velvetleaf huckleberry)  
*Vaccinium oxycoccos* (small cranberry)  
*Vaccinium vitis-idaea* (lingonberry)  
*Valeriana uliginosa* (mountain valerian)  
*Vallisneria americana* (American eelgrass)  
*Verbascum thapsus* (common mullein)  
*Veronica officinalis* (common gypsyweed)  
*Veronica scutellata* (skullcap speedwell)  
*Veronica serpyllifolia* (thymeleaf speedwell)  
*Viburnum cassinoides* (*Viburnum nudum* var. *cassinoides*)  
*Viburnum lentago* (nannyberry)  
*Viburnum recognitum* (*Viburnum dentatum* var. *lucidum*)  
*Viburnum trilobum* (*Viburnum opulus* var. *americanum*)  
*Vicia cracca* (bird vetch)  
*Vicia sepium* (bush vetch)  
*Viola adunca* (hookedspur violet)  
*Viola cucullata* (marsh blue violet)  
*Viola pallens* (*Viola macloskeyi* ssp. *pallens*)  
*Viola septentrionalis* (northern blue violet)